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The MES logo features the Maryland Shield below a drawing of a specimen of Euphydryas phaëton (Drury), the Baltimore checkerspot [which became the official insect for the state of Maryland through the efforts of many members of this Society], with its generic name above and its specific epithet below (both in capital letters), all on a pale green field; all these are within a double-bordered yellow ring bearing the message ". Maryland Entomological Society . 1971 ." The two borders of this yellow ring are red.

A New Subspecies of Incisalia henrici (Grote and Robinson) (Lepidoptera: Lycaenidae) from the Outer Banks of North Carolina

Harry Pavulaan[1]

ABSTRACT. A new subspecies of Incisalia henrici (Grote & Robinson) is herein described from resident populations of this butterfly occurring on the North Carolina Outer Banks. These populations contain a high predominance of adult individuals which display a combination of three distinct greenish ventral characters, and one dorsal character.

INTRODUCTION

Populations of Incisalia henrici (Grote & Robinson)from eastern North Carolina and the Chesapeake Bay region of Maryland, Delaware and Virginia display a tendency for development of greenish characters on the ventral wing surfaces. This tendency is strongest in populations along the North Carolina Outer Banks, Which is herein designated as the type locality (TL) of the new subspecies Incisalia henrici viridissima described below. There, strongly-greenish phenotypes predominate. A smaller percentage of TL adults, which I consider to be intermediates to nominotypical I. h. henrici, display one or two of three basic greenish characters. A very small number of brown adults resembling the nominotypical form do occur here, but there are still minor differences.

HISTORY

The greenish I. henrici phenotype went virtually unnoticed until 1983, prior to which there was no literature reference. Even now, very little has appeared in the literature about greenish characters in I. henrici. Samuel Gifford was the first person known to have studied the Outer Banks population of I. henrici. Gifford observed this population during the years 1974-1980, but did not publish his findings for several years. In a paper on the biology of several hairstreaks (Gifford & Opler, 1983), the authors very briefly stated: "Individuals of the Hatteras Island population had a greenish cast ventrally, and might eventually be described as a separate subspecies...". Later, in Opler & Krizek (1984), it was briefly mentioned that "in populations [of I. henrici] from North Carolina's Outer Banks most individuals have strong green highlights ventrally". Subsequent inquiries with various sources led me to conclude that Samuel Gifford had been the only real authority on the green phenotype, though there was no indication that he intended to publish a paper on the subject. Gifford was the source of most I. h. viridissima specimens in several collections which I examined.

This article was critically reviewed by two annonymous peers and at least one member of the MES Editorial Committee.

[1] 494 Fillmore Street, Herndon, VA 22070.

DIAGNOSIS

A combination of four characters distinguish I. h. viridissima from the nominotypical subspecies and all other described subspecies and forms of I. henrici: a nearly uniform dark (fuscous) brown dorsum; ventral basal green dusting; ventral postmedian greenish tint; and ventral postmedian greenish iridescence. These characters were not specified in the original descriptions of nominotypical I. henrici (Grote and Robinson 1867), subspecies I. h. margaretae (dos Passos 1943), I. h. solata (Cook and Watson 1909), or I. h. turneri (Clench 1943), and have not been mentioned as occurring in another as yet undescribed subspecies from the southeastern U.S. (Gatrelle 1985).

DESCRIPTION

All color names in parentheses are from: "Color Standards and Color Nomenclature" (Ridgeway 1912).

Incisalia henrici viridissima, new subspecies (Figs. 1 and 2).

Wing shape, tails, scalloping as in nominotypical henrici, though tails average about 0.5 mm longer. Above (Fig. 1), uniform dark (fuscous) brown in the vast majority of individuals, often with a brassy sheen, shaded very diffusely over the postmedian portion of the primaries in some females, and in rare individuals also on the secondaries, with a faint rusty (Sanford's) brown. Also, in the submarginal portion of secondary wing cells CuA1 and CuA2, before the anal angle of the hindwing, a bright rusty (Sanford's) brown patch in both sexes. Rarely, this rusty area extends into cell M3, more likely in females. The fringes on the primaries and on the foreward portion of the secondaries are light, rarely whitish, but more often pale brownish or greyish, and occasionally very dark, and are interrupted at the veins which are tipped with blackish. Toward the anal angle of the hindwing, the fringes are usually dark.

The venter is characterized by three major green characters in combination: (1) The variable presence of green dusting on the basal half of the secondaries. Less frequently, this dusting is also present on the primaries, along the costal portion of the basal half. Rarely, the entire underside is covered with greenish peppering. (2) A greenish tint along the postmedian half of the secondaries and also in the subapical portion of the primaries. (3) Greenish iridescence along the postmedian half of the secondaries and also in the subapical portion of the primaries. A full description follows:

Beneath (Fig. 2), the primaries are of a variable (snuff to sayal) brown from the base outward to the long dark brown postmedian transverse line at the apical third running over the veins and extending from the costal margin to CuA2. There is also a very small dark brown transverse line or dash about halfway along the costal margin of the wing extending from vein M1 inward to vein M3, being curved very slightly, with concave side facing toward the base. Outside of the postmedian transverse line, the submarginal third of the wing is paler toward the apical portion, being of a greenish (variably serpentine

green, yellowish citrine, oil green, lettuce green, warbler green or pyrite yellow) brown. There is a row of subdued dark brown submarginal spots, usually present and centered in cells M2, M3, and CuAl, but variably present and fading in the cells toward costal and inner margins. There is also a row of subdued dark brown marginal spots, usually present in cells CuA2 to M2, but fading toward the apex. The fringes are as on the upper surface.

In the secondaries, the base is dark (maroon to bay) brown, paler (like the adjacent primaries) along the lower costa, and limited outwardly by the median line; this portion of the wing is sparsely clothed with pale and longer hairs, except on the costal region outwardly, and is very variably peppered with green scales. The median line is essentially the edge of the basal dark area, free of green scaling, and appears dark (maroon to bay) brown. It is shaped as in nominotypical I. henrici, being very jagged, and is succeeded by faint white scales. The white scales increase to become small white median dashes across cell Sc+Rl and cell lA+2A. Outside the marginal line, in the inner postmedian area, the wing varies from a pale (Dresden brown, light brownish-olive or Saccardo's umber) brown to greenish (variably serpentine green, yellowish-citrine, oil green, lettuce green, warbler green or pyrite yellow) brown. Extreme individuals are strongly (olive-yellow to light yellowish-olive) green. This greenish area displays a green iridescence, strongest in natural sunlight. There is a jagged postmedian row of undulating semilunate, black, interspace crescents as in nominotypical I. henrici, edged inwardly by very faint white scales which are often obliterated. Beyond these crescents, toward the apical third, the submarginal space is quite variably pale (snuff to sayal) to dark (maroon to bay) brown to greenish as in the inner postmedian area. In the remaining (tornal) two thirds of the submarginal space is a hoary, greyish, violet blue hue. Toward the inner part of this hoary zone, is a subdued pinkish shade which parallels the postmedian crescents, and is strongest immediately outside each crescent. There is a narrow, interrupted, black internal marginal line, bordered in the extreme external margin by a dark (maroon to bay) brown line. The fringes are as on the upper surface.

Interestingly, the ventral green coloration on the outer third of the wings seems to fade somewhat with age in most specimens, though the basal green scales of the secondaries retain color, and the postmedian iridescence remains. Optimally, one should examine specimens under natural sunlight to view the full effect of the green characters in combination {individuals observed resting in full sunlight often appear fully-green, occasionally not unlike the Olive Hairstreak, Mitoura grynea (Hübner) owing to strong irridescence in natural light]. Incandescent and flourescent lighting do not reveal true color as seen in nature. Specimen dispatching and relaxing fluids generally destroy all green characters. Thus, the disappointment of several colleagues who claim to have captured striking viridissima-like individuals in blend zone locations, only to find their voucher specimens' green features quite subdued under artificial light.

The head and body are dark (fuscous) brown, with a covering of long hairs. This covering of hairs is generally thickest on the head and thorax, and more sparse on the abdomen. The antennae are black, strongly annulated with white rings, and the club is dark (fuscous) brown to blackish. The palpi are greyish-brown to blackish. The eyes are

very narrowly margined with white. The legs are greyish, variably ringed with white. Forewing length along the costal margin ranges from 11.0 mm to 15.0 mm, average length is 13.0 mm, with 50% of all examined specimens measuring 13.0 mm.

Intermediates from the Outer Banks and blend zone generally fit the description of I. h. viridissima, except that only one or a combination of two of the green characters are expressed. Fully-brown individuals from the Outer Banks resemble I. h. viridissima in all respects, except that they lack any of the three green characters. These are slightly larger than northern (nominotypical) and inland populations, are somewhat paler beneath, generally dark above (compared to nominotypical populations which display variable degrees of dorsal rusty brown), and have minutely longer tails.

ETYMOLOGY

The subspecies name comes from Latin "viridissima", which means "the greenest of all" I. henrici. If deemed necessary for future purposes, I propose that the name "Greenish Henry's Elfin" be applied as the common name. This name is currently widely used by local lepidopterists.

RANGE

The type locality (TL) of I. h. viridissima is Bodie Island lighthouse, near Oregon Inlet, Nag's Head, Dare Co., NC (Fig. 5). This location lies within Cape Hatteras National Seashore. There are also records from the Frisco and Buxton Woods area north to Kitty Hawk, all on the Outer Banks. This small area comprises what I consider the range of true subspecies I. h. viridissima, though it may eventually be found that similarly high percentages of the green phenotype may occur in yet undocumented populations further north and south along the barrier beaches and also inland at some locations.

There is a broad inland blend zone (Fig. 5) in which most individuals are predominantly brown, somewhat resembling nominotypical I. h. henrici (Fig. 3), but differing from the nominotypical phenotype in having a somewhat lighter brown ventral ground color, a darker dorsal surface color [generally lacking the extensive orange-brown coloration found on the dorsal wing surfaces of northern (I. h. henrici) and midwestern (I. h. turneri) populations], and in being slightly larger. In these populations, individuals intermediate to I. h. viridissima are frequent to varying degrees (usually <10% in examined series, but as high as 50% in populations from Anne Arundel Co., MD), displaying one or two of the greenish characters. There is also a presence of strongly greenish viridissima-phenotype individuals (usually <5% in examined series) that are virtually indistinguishable from TL specimens. The currently-known distribution of the I. h. viridissima phenotype and intermediates on the mainland extends west only to Craven Co., NC, north around the west side of the Chesapeake Bay over to the WV and western MD panhandles, into southern

Maryland, the Delmarva Peninsula, and southern NJ. Scott (1986, plate 332) shows a photograph of what appears to be a slightly intermediate specimen from central NJ.

Beyond the blend zone, from PA and northern NJ, northward into New England, and westward into WV, populations are nominotypical I. h. henrici. Specimens from much of VA and NC were not available for examination. Populations from southern NC, south into SC and GA, over into MS (Mather 1958) constitute a weak southern subspecies (Gatrelle 1985), which grades somewhat into the FL subspecies I. h. margaretae (dos Passos 1943), but also displays some intrinsicly unique characters. This subspecies is characterized mainly by less contrast in ventral markings, and by a wine-brown ventral ground color. The dorsum is primarily dark greyish brown. Specimens that I have seen from coastal SC (Fig. 4) are somewhat larger than I. h. viridissima and nominotypical I. h. henrici specimens, and are also larger than I. h. margaretae specimens which I have examined. The tails are intermediate in length between I. h. margaretae and all other I. h. henrici populations.

The midwestern subspecies, I. h. turneri is also a very weak subspecies, based primarily on the strongly orange-brown dorsum. Turneri-like individuals are frequent throughout the Appalachians and as far east as Rhode Island and eastern Ontario. Populations in the southern Appalachians and states west to the Mississippi River cannot be considered true nominotypical I. h. henrici, but include a broad range of intermediates and extremes ranging from nominotypical I. h. henrici to I. h. turneri, with some individuals resembling the unnamed southeastern subspecies. Interestingly, a series of specimens from the extreme eastern Ozarks of Missouri appears uniformly much more like nominotypical I. h. henrici than any I have seen outside of the northeastern states. These are generally very small with a predominantly dark dorsum and a dark greyish-brown venter.

Reports of occasional viridissima-like specimens in I. h. turneri populations in the midwest (Kral, Wright, pers. comm.) with greenish venters, need confirmation. There is nothing in the literature regarding greenish specimens from the midwest, nor have I personally seen any specimens in examined collections. Any viridissima-liked specimens in I. h. turneri (strongly orange-brown dorsum) populations would not be considered I. h. viridissima unless their dorsum were dark. If viridissima-like individuals can be confirmed to occur in midwestern populations, then we would see an interesting situation parallel to that of the Falcate Orange Tip, Anthocharis midea (Hübner). The nominotypical population, A. m. midea, occurs in coastal SCa and GA, with a very narrow blend zone inland to subspecies A. m. annickae (dos Passos and Klots), which essentially occupies the remainder of the species' range from New England west to Nebraska and south to Texas. However, individuals strongly resembling nominotypical A. m. midea appear in populations in Nebraska and Misssouri (personal observations), occasionally comprising the majority of individuals at some locations.

HABITAT

The type locality colony is located in a maritime thicket habitat at the leeward fringe of a large stand of mature loblolly pine (Pinus taeda). These tall pines provide a natural windbreak, sheltering the habitat from the relentless pruning effect of onshore winds and salt spray, which would otherwise limit the height of associated trees and shrubs to a low, dense, windswept canopy. Small butterflies such as I. henrici are not known to inhabit such windswept environments. The trees and shrubs along the lee side of the protecting pines, however, are taller than in the dense windswept shrub thicket which occurs closer to the ocean. Some of the resident shrubs have attained small tree size, thus contributing to the windbreak effect. Ocean breezes are thus tempered in the primary habitat, allowing for considerable solar radiation in the spring. This warming effect is most perceptible during cool, windy spring mornings in sunlit places along the pine forest edge, and in clearings in the protected maritime thicket.

Associated prominent woody plants in this protected maritime thicket are: eastern red cedar (Juniperus virginiana), highbush blueberry (Vaccinium corymbosum), American holly (Ilex opaca) and black cherry (Prunus serotina), which are found primarily in open areas. Yaupon holly (Ilex vomitoria) and waxmyrtle (Myrica cerifera) both occur in open areas, but also beneath the loblolly pine canopy, and form a dense perimeter along the pine forest fringe, most noticeable along the maintenance-area service road. Loblolly pines also grow out in the thicket, as smaller trees. Greenbrier (Smilax spp.), forms impenetrable tangles in all areas, which impede movement of larger animals.

Additional specimens were collected over several years by various people in separate wooded habitats on the Outer Banks. Numerous specimens have been taken at Frisco and Buxton Woods (in areas outside the National Seashore) where I. vomitoria is common in areas bordering the forest habitat, but no large concentrations of the butterfly have been found. Part of Buxton Woods lies within the National Seashore, while part remains unprotected and may be subject to increasing development pressure in future years. Additionally, a small population exists in what I consider "mainland" habitat in the barrier island woods near the Wright Memorial Bridge.

On the mainland, I. h. viridissima blend zone populations are associated with dense stands of American holly (Ilex opaca) in mature pine or hardwood (oak-dominated, mainly) woodland. These populations can be extremely abundant during some years, almost swarming about host trees in some locations.

HOSTS

Yaupon holly (I. vomitoria) - Gifford and Opler (1983) reported that the Hatteras Island population utilizes only yaupon holly, ovipositing adjacent to the midrib on the upper surface of the previous year's leaves. In 1991, I found one ovum on the upperside midrib of an older leaf, near an unopened new bud. The southeastern subspecies also utilizes yaupon holly along the South Carolina coast.

American holly (I. opaca) - Gifford and Opler (1983) reported that only American holly was used on Roanoke Island. However, in 1991, I discovered a small tree at the TL that was frequented by several females. Close examination revealed that several ova had been deposited on prominent leaf buds at the tree top, as well as on upper leaf surfaces (old leaves) on prominent upper branches. One ovum was found on a leaf undersurface, near the leaf edge. American holly is also the main host of blend zone populations around the Chesapeake Bay and also of nominotypical I. h. henrici populations at Sandy Hook and Batsto, NJ, and in southern RI. Gatrelle (1985) reported American holly as the hostplant of the southeastern subspecies, in South Carolina.

Two captive females from the barrier strip woods near the Wright Memorial Bridge were confined on a sleeved American holly in lab conditions. Numerous ova were deposited over a 14-day period, mainly on fresh leaf buds, but also on leaf stems, end twigs, and on the previous year's leaves. After two weeks of feeding on American holly, the larvae were transferred to young redbud (Cercis canadensis) leaves, due to a shortage of available holly in prime feeding state, in the area of my residence in central Maryland at that time. They only nibbled on the leaves at first, finally accepting them after about two days. The larvae developed normally and formed pupae (100+), which were refrigerated for approximately 5 months. After removal from refrigeration, only 5 adults emerged, two of them aberrated. The remaining pupae did not break diapause and were returned to refrigeration for an additional 3 months, after which time all were determined to be dessicated. Wright (pers. comm.) suggested that perhaps C. canadensis may not be an optimum foodplant for I. h. viridissima, resulting in the high mortality rate in the rearing experiments. Further work is needed here. American holly-associated females from Prince George's Co., MD (western Chesapeake blend zone) would not oviposit on redbud in captivity, but freely oviposited on American holly. Redbud is the predominant host for nominotypical I. h. henrici populations in the central and southern Appalachian region, and for midwestern I. h. turneri. Populations centered in the eastern West Virginia panhandle, which contain a small percentage of viridissima-like individuals and intermediates, also seem to be associated with redbud. Very little is known about host preferences around Philadelphia, PA, the species' TL. Redbud may be the primary host there. Shapiro (1966) reported wild plum (Prunus pennsylvanica) as a host in parts of eastern PA. This host occurs in moderate numbers in the Philadelphia area, though Wright (pers. comm.) felt that plum is rarely used in Pennsylvania.

HABITS

These adults are among the first butterflies to emerge in early spring, generally flying with the Spring Azure, Celastrina ladon (Cramer). The earliest records from the Outer Banks are March 24 (Ferguson), but worn condition of some adults from around this time indicate that the flight period may begin sometime in mid-March, with numbers peaking around April 1. Latest records are from April 17 (Gifford). Yearly emergence patterns are likely to be highly dependent on varying weather conditions.

Earliest emergence in inland (blend zone) populations, immediately west of the Outer Banks, definitely occurs in early to mid-March (Sullivan, pers. comm.). To the north, in the broad blend zone around the Chesapeake Bay, the first emergences occur in early April, with numbers peaking in mid-April, but this situation is also highly dependent on seasonal conditions from year to year.

The daily flight period at the TL, depending on weather, begins at about 10:00 AM (11:00 AM Daylight Savings Time). Peak activity continues for about two hours, then suddenly drops off. Adults are closely associated with host trees or shrubs in the protected sunlit microclimate of the maritime thicket, usually conducting most activity on, about, or near the hosts. At the TL, I. h. viridissima adults generally prefer abundant stands of yaupon holly along the forest fringe, but also occur out in the protected maritime thicket where larger host shrubs occur in bunches. Males and females perch on prominent branch ends, flying out at other individuals and engaging in territorial aerial displays, then returning to the same perch or a nearby one. The major inland host, American holly, is not very common at the TL, but females have been observed ovipositing on upper branches of one of these trees here. Adult I. henrici avoid the shade of the loblolly pine canopy, but an occasional individual has been seen frequenting sunlit hosts just inside the loblolly pine canopy. Observations recorded in Cape May Co., NJ (blend zone), indicate that adults revert to nectaring and mating activity about flowering highbush blueberry (V. corymbosum) shrubs in the early afternoon. Adults have been found resting on pines near Buxton Woods in late afternoon, but were otherwise inactive (Ferguson, pers. comm.).

In mainland (blend zone) locations, I. henrici adults are active in dense understory stands of host American hollies in forested habitats during the early spring, when the predominant oaks have not yet leafed out. Here, American holly enjoys full sunlight beneath the leafless early-spring forest canopy.

Adults have been observed nectaring on flowers of black willow (Salix nigra) (Zeligs, pers. comm.), highbush blueberry (V. corymbosum), sassafras (Sassafras albidum), wild black cherry (P. serotina), and also reportedly other coastal Prunus spp. One adult has also been sighted imbibing moisture from wet sand (Grooms, pers. comm.).

DISCUSSION

I believe the greenish characters found in I. h. viridissima are an early-stage evolutionary adaptation to the evergreen holly forest habitat in the mid-Atlantic coastal region. This would seem to support the phyletic gradualism model of macroevolution, in which established populations slowly change over a long period of time, adapting to environmental influences. Individuals with strong greenish coloration might gain protective advantage by blending imperceptibly against a background of green holly leaves in early spring. Inland populations, which have a predominantly brown venter and feed primarily on redbud (Cercis canadensis), do not stand to gain from such coloration against the stark leafless dark brown branches of their early-spring forest habitat. Theoretically, the green characters in holly-feeding populations of I. h. henrici may have been selected over time, providing an effective measure of camouflage.

Figure 3. Left side: I. h. henrici, ventral view, male, 29-IV-88, West Kingston, Washington Co., RI; Right side: I. h. henrici, ventral view, female, 29-IV-88, West Kingston, Washington Co., RI.

Figure 4. Left side: I. henrici, southeastern subspecies, ventral view, male, 27-III-86, near Beaufort, Beaufort Co., SC; Right side: I. henrici, southeastern subspecies, ventral view, female, 26-III-86, near Beaufort, Beaufort Co., SC.

Figure 1. Left side: I. h. viridissima, dorsal view, holotype male, 3-IV-91, Bodie Island lighthouse, Cape Hatteras National Seashore, near Nag's Head, Dare Co., NC; Right side: I. h. viridissima, dorsal view, allotype female, 3-IV-91, Bodie Island lighthouse, Cape Hatteras National Seashore, near Nag's Head, Dare Co., NC.

Figure 2. Left side: I. h. viridissima, ventral view, holotype male, 3-IV-91, Bodie Island lighthouse, Cape Hatteras National Seashore, near Nag's Head, Dare Co., NC; Right side: I. h. viridissima, ventral view, allotype female, 3-IV-91, Bodie Island lighthouse, Cape Hatteras National Seashore, near Nag's Head, Dare Co., NC.

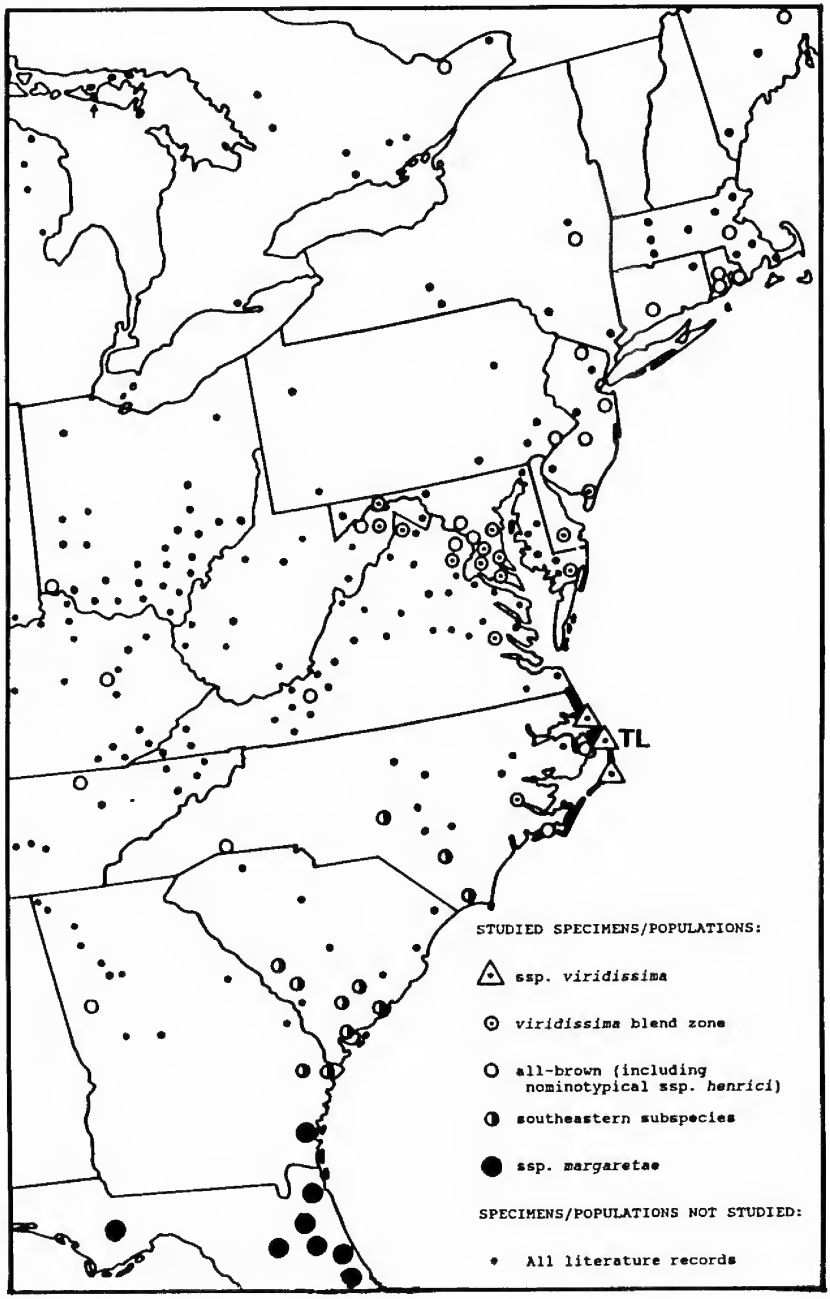

Figure 5. Distribution of Incisalia henrici phenotypes in eastern North America showing collection locations of specimens examined. Dots showing literature records from the eastern United States are from Opler (1983). Dots showing literature records from eastern Canada are from Holmes, et. al. (1991).

Table 1. Genereal distribution of four eastern Incisalia henrici phenotypic characters. These characters are explained in the preceding text. The four general phenotypes were: gr = viridissima; int = intermediate to viridissima; br = "all-brown" phenotypes (including nominotypical I. henrici); tu = I. turneri; and se = southeastern subspecies.

State	County	No. of Specimens of each Phenotype Examined			
CT	New Haven Co.		31 br		
DE	Sussex Co.		5 br	3 int	
Wash., DC	- N/A -			2 br	
GA	Bryan Co.	4 se			
	Chatham Co.	3 se			
	Coweta Co.	1 se			
KY	Powell Co.		1 br		
MA	Norfolk Co.		28 br		
MD	Allegany Co.		8 br	1 int	
	Anne Arundel Co.	95 br	36 int	15 gr	
	Calvert Co.		9 br	1 int	
	Charles Co.		40 br	3 int	
	Montgomery Co.	1 br			
	Prince Georges Co.		83 br	4 int	11 gr
	St. Mary's Co.			1 int	1 gr
	Worchester Co.				2 gr
ME	Penobscot Co.		4 br		
MO	St. Francois Co.		9 br		
MS	Hinds Co.	1 se	1 br		
NJ	Burlington Co.		1 br		
	Cape May Co.		31 br	13 int	9 gr
	Monmouth Co.		8 br		
	Passaic Co.		10 br		
NY	Albany Co.		6 br		
NC	Bladen Co.	1 se			
	Brunswick Co.	3 se			

	Carteret Co.		1 br			
	Craven Co.		1 br	1 int		
	Dare Co. (Outer Banks)	1 se	8 br	14 int	55 gr	
	Dare Co. (mainland)		1 br			
	Moore Co.	4 se	5 br			
	Polk Co.		1 br			
OH	Brown Co.		1 br			
	Hamilton Co.		5 br			
	Lawrence Co.		1 br			
Ont., CAN	Ottawa					6 tu
PA	Philadelphia		1 br			
RI	Kent Co.		1 br			
	Newport Co.		1 br			
	Washington Co.	9 br				
SC	Aiken Co.	100% se				
	Barnwell Co.	100% se				
	Beaufort Co.	100% se				
	Charleston Co.	100% se				
	Colleton Co.	100% se				
	Dorchester Co.	100% se				
	Orangeburg Co.	1 se				
TN	Scott Co.		3 br			
VA	Fairfax Co.		1 br			
	Frederick Co.		3 br	2 int	1 gr	
	Montgomery Co.	7 br				
	New Kent Co.		28 br		1 gr	
	Prince William Co.		12 br	4 int	1 gr	
WV	Hampshire Co.		10 br	1 int		
	Mineral Co.		2 br			

SPECIMENS EXAMINED

Specimens from the following locations (state, county) were examined to determine th distribution of greenish characters (Table 1). KEY: br = nominotypical and near-nomino typical (all-brown) phenotypes; int = intermediate forms present, displaying one or two o the three greenish characters; gr = I. h. viridissima phenotype displaying all three greenis characters; se = southeastern (brown) subspecies; tu = subspecies I. h. turneri (brown). Incisalia h. margaretae has been omitted from this study (though known counties are mapped for reference purposes), as the distribution is well-documented. Numbers indicate actual numbers of specimens counted. Percentage figures indicate a large number of specimens (20+) were examined from a single location, all of which were of similar coloration, generally brown phenotypes. Data for some South Carolina and Georgia counties also include determinations of the southeastern phenotype from literature (Gatrelle 1985) and others (Gardner, pers. comm.). Note: specimens collected by the present author at the TL were done under permit (ref. # A 9015) from the U.S. Dept. of the Interior, Cape Hatteras National Seashore. Copy available upon request.

HOLOTYPE, ALLOTYPE, AND PARATYPE SPECIMENS

HOLOTYPE: male (Figs. 1 & 2), TL = Bodie Island Lighthouse, Cape Hatteras National Seashore, near Nag's Head, Dare Co., NC, 3-IV-1991, Harry Pavulaan, deposited in USNM. **ALLOTYPE**: female (Figs. 1 & 2), Bodie Island Lighthouse, Cape Hatteras National Seashore, near Nag's Head, Dare Co., NC, 3-IV-1991, Harry Pavulaan, deposited in USNM. **PARATYPES**: Paratypes # 1 - 3: 3 males, Bodie Island Lighthouse, Cape Hatteras National Seashore, near Nag's Head, Dare Co., NC, 3-IV-1991, Harry Pavulaan, deposited in USNM. Paratype # 4: female, ex-ovum from Nag's Head, Dare Co., NC; ovum on 12-IV-1991, on I. opaca, larva reared on I. opaca (2 weeks) and C. canadensis (2 weeks), emerged 5-X-1991, collected/reared by Harry Pavulaan. Paratypes # 5 - 8: 4 males, Kitty Hawk, Dare Co., NC, 31-III-85, Harry Pavulaan. Paratypes # 9 - 17: 9 females, Kitty Hawk, Dare Co., NC, 31-III-85, Harry Pavulaan (Note: paratype # 17's original green characters were obliterated due to greasing of wings, and subsequent deagreasing with Naptha lighter fluid). Paratypes # 18 - 25: 8 males, Kitty Hawk, Dare Co., NC, 28-III-89, Harry Pavulaan. Paratypes # 26 - 31: 6 females, Kitty Hawk, Dare Co., NC, 28-III-89, Harry Pavulaan. Paratype # 32: male, brown form, Kitty Hawk, Dare Co., NC, 28-III-89, Harry Pavulaan. Paratype # 33: male, Kitty Hawk, Dare Co., NC, 7-IV-93, Harry Pavulaan. Paratype # 34: male, ex-ovum from Nag's Head, Dare Co., NC, ovum on 31-III-91 on I. opaca, larva reared on I. opaca (2 weeks) and C. canadensis (2 weeks), emerged 20-X-91, collected/reared by Harry Pavulaan, sent to John Emmel. Paratype # 35: male, ex-ovum from Nag's Head, Dare Co., NC, ovum, 31-III-91 on I. opaca, larva reared on I. opaca (2 weeks) and C. canadensis (2 weeks), emerged 3-X-91, collected/reared by Harry Pavulaan, sent to John Emmel. Paratypes # 36 - 43: 8 males, Buxton, Dare Co., NC, 14-IV-79, collection of Joseph Zeligs. Paratypes # 44 - 45: 2 females, Buxton, Dare Co., NC, 14-IV-79, collection of Joseph Zeligs.

Paratypes # 46 - 47: 2 males, Nag's Head, Dare Co., NC, 31-III-93, collection of Bill Grooms. Paratypes # 48 - 61: 14 males, Frisco, Dare Co., NC, 25-III-75, Douglas C. Ferguson, CMNH collection. Paratype # 62: female, Frisco, Dare Co., NC, 25-III-75, Douglas C. Ferguson, CMNH collection. Paratype # 63: sex undetermined (abdomen missing), Frisco, Dare Co., NC, 25-III-75, Douglas C. Ferguson, CMNH collection. Paratype # 64: male, right-side aberrant, Frisco, Dare Co., NC, 25-III-75, Douglas C. Ferguson, CMNH collection. Paratype # 65: female, Frisco, Dare Co., NC, 24 -III-75, Douglas C. Ferguson, CMNH collection. Paratypes # 66 - 68: 3 males, Frisco, Dare Co., NC, 26-III-75, Douglas C. Ferguson, CMNH collection. Paratype # 69: male, Frisco, Dare Co., NC, 28-III-75, Douglas C. Ferguson, CMNH collection. Paratype # 70: male, brown form, Frisco, Dare Co., NC, 28-III-75, Douglas C. Ferguson, CMNH collection. Paratype # 71: female, Hatteras Island, Dare Co., NC, 17-IV-71, Sam Gifford, CMNH collection. Paratypes # 72 - 73: 2 males, Hatteras Island, Dare Co., NC, 17-IV-71, Sam Gifford, CMNH collection. Paratypes # 74 - 76: 3 males, brown form, Hatteras Island, Dare Co., NC, 17-IV-71, Sam Gifford, CMNH collection. Disposition of Holotype, Allotype, and Paratypes # 1 - 3 as stipulated in U.S. Dept. of the Interior collecting permit (ref. # A 9015).

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Environmental Exposure to Cu^{2+} in *Anax junius* (Drury) Naiads (Odonata: Aeshnidae) Causes Disruption in Enzymatic Mobilities[#]

S.J. Harrison [1] and A.P. Platt [2]

Abstract. Using starch-gel electrophoresis, 12 different enzymes were examined in *Anax junius* (Drury) naiads after exposure to 0, 1, and 10 mg $CuSO_4$/l. The naiads were collected from an unpolluted lentic environment. Different gel buffer systems appropriate for specific enzymes were utilized. Mortality was 100% in naiads exposed to 10 mg $CuSO_4$/l. Differences in activity were observed in six of the 12 enzymes in copper-treated animals compared to controls.

Introduction

Copper has been reported to be disruptive to many enzymes, usually by suppression of activities, but occasionally by enhancement (Dixon and Webb 1964; Owen, Jr. 1981; 1982; Steinkühler 1991). Hodson, *et al.* (1979), reported a number of studies in which copper inhibited enzyme activities in fish. Two important observations in their compilation were: 1) that more inhibition occurred in vitro than in vivo, suggesting that either copper was unable to enter certain cells, or that a mechanism existed for counteracting it in vivo; 2) that more inhibition of δ-aminolevulinic acid dehydrase activity occurred after four days of exposure than after 14 days suggesting that tolerance may develop with chronic exposure. Other examples of resistance to chronic exposure to copper have been reported (Brown 1976; Winner and Gauss, 1986). Feric iron ions (Fe^{3+}), cupric ions (Cu^{2+}), and oxygen (O_2), in the presence of an appropriate electron donor, were found to catalyze oxidative modification of proteins by causing reactions or secondary reactions at metal-binding sites (Statman 1990). Dixon and Webb (1964) found that extremely small traces of copper in water which had not been highly purified resulted in instability of enzymes and loss of activity during recovery through dialysis. They noted a high affinity for copper in enzymes, and that all traces may be taken up even from a very large volume of water. In contrast, copper may also be an activator or co-factor for some enzymes (Wolfe 1986). Harris, et al. (1980) found that lysil oxidase activity was either diminished or eliminated in the absence of copper, however the activity returned when copper was restored to the diet. Similar effects have been observed with other metals. Milstein (1991) found that phosphogluco-mutase was activated by Mg^{2+} ions. Non-metallic compliments have also been known to activate various enzymes (Friedl, *et al.* 1989).

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[1] Towson State University (TSU), Biology Department, 320 Smith Hall, Towson, MD 21204-7097, USA. current address: The Johns Hopkins Asthma & Allergy Center (JHAAC), Division of Pulmonary & Critical Care Medicine, Rm. 4B.72, 5501 Hopkins Bayview Circle, Baltimore, MD 21224-6821, USA.
[2] University of Maryland Baltimore County (UMBC), Department of Biological Sciences, 1000 Hilltop Circle, Baltimore, MD 21250, USA.

Key words: Anisoptera, *Anax junius*, starch-gel electrophoresis, $CuSO_4$, Cu^{2+}, enzymes.

Few studies have been done on enzymatic functions in the odonates. Meyer *et al.* (1986) found a reduction of activities of succinic dehydrogenase, and cytochrome c reductase in odonate naiads exposed to lead. Dierickx (1984) studied the activity of gluthathione S-transferase in aquatic macroinvertebrates subjected to various organic micropollutants. He found an activity level in odonates that was six times higher than that of other organisms. Since gluthathione S-transferase is a detoxification enzyme, his data may explain why dragonflies are more resistant to pollution than are some other aquatic insects. An electrophoretic analysis by Schott and Brusven (1980) showed different enzymatic activities related to a temperature gradient in Zygoptera. They found differences in four enzymes: glucose-6-phosphate dehydrogenase, lactate dehydrogenase, leucine aminopeptidase, and tetrazolium oxidase. Malate dehydrogenase and esterase showed no such differences. Benton and Guttman (1990) found that in an electrophoretic analysis of Ephemeroptera exposed to copper, time to death could be correlated with certain polymorphic loci of several enzymes including: glucose phosphate isomerase, phosoglucomutase, and malate dehydrogenase.

Cupric sulfate ($CuSO_4$) is commonly used as an aquatic algacide (Hinman, *et al.* 1942; McBrien 1980), and is known to be toxic in varying degrees to non-target organisms (Nor 1987). While some studies have examined the effects of $CuSO_4$ on non-target organisms (McIntosh 1974), the effects of cupric ions on odonate naiads is not well known. The present electrophoretic analysis of various enzymes in Anisoptera naiads was conducted to determine whether exposure to $CuSO_4$ causes variation in enzymatic mobilities, thus indicating that metabolic impairments may be occurring in natural populations of these organisms.

Materials and Methods

Twenty-seven *Anax junius* (Drury) naiads, Family: Aeshnidae, commonly called the Green Darner (Borror 1963) were taken from among cattails, *Typha latifolia* (L.), in a temporary pond on the University of Maryland Baltimore County (UMBC) campus in late Autumn. The species was determined using standard dichotomous keys (Needham and Needham 1962; Pennak 1978; Borror, *et al.* 1981; Merritt and Cummins 1984).

The naiads were divided into three groups of nine and were introduced into aqueous solutions consisting of 0, 1, or 10 mg $CuSO_4$/l filtered pond water. The pond water was tested for ambient Cu^{2+} using a HACH DR-EL spectrophotometer after passing it through a .22 μ filter. Only a negligible amount of copper registered. Controls consisted of animals which had been exposed to filtered pond water only. The pond water in each group was adjusted to pH 7.0 to facilitate comparison, and to eliminate combined effects of pH and metal ions which are known to occur (Robinson and Deano 1986).

The naiads were starved during the course of the experiment to prevent possible gut-content interference in the assays. When animals were dead, or nearly so, (*i.e.*, when their response to agitation was so sluggish that it was difficult to determine whether they were moving on their own or simply being carried along by water movements resulting from the stimulus), they were frozen at -70°C. After five days, a time chosen to prevent artifacts in enzyme depletion (Terra *et al.* 1990), all remaining animals were frozen at -70°C and stored until the gels could be run.

Enzymatic mobilities were determined using horizontal starch-gel electrophoresis based on the methods of McCracken (1976) and May, *et al.* (1979). Three different gel systems were used: C (Clayton and Tretiak 1972), R (Ridgeway, *et al.* 1970), and 4 (Selander, *et al.* 1971). The particular gel system chosen for each enzyme was based on those used by Brussard (1985); [see Harrison (1993) for exact methods]. The gels were loaded so that each lane represented a single animal. To determine if the copper effect was biochemical or metabolic in origin, four controls in the aeshnid gel were treated by mixing 1 ml of the 10 mg/ml $CuSO_4$ into the extraction preparation immediately prior to running the gel.

All 27 animals were tested for each of the following enzymes: Aldolase (ALD); Esterase (EST); Galactosaminidase (GAM); Glucosekinase (GK); Glucosephosphate isomerase (GPI); α-Glycerophosphate dehydrogenase (α-GPD); Isocitrate dehydrogenase (IDH); Malate dehydrogenase$^+$ (MDH^+); Malate dehydrogenase$^-$ (MDH^-); Peptidase (PEP); Superoxide dismutase (SOD); and Xanthine dehydrogenase (XDH).

Results & Discussion

The results of this study demonstrated that Odonata naiads, when exposed to cupric ions, exhibited significant alteration in enzymatic activity. All of the naiads in the 10 mg/ml Cu^{2+} concentration died within two days, while the animals in the 1 mg/ml Cu^{2+} concentration survived to the end of the experiment although three of them were becoming quite sluggish. Benton and Guttman (1990), in their study of mayflies, found an 88% mortality rate after 126 hr when their animals were exposed to 1.6 mg Cu/l. Since the Odonata are generally recognized as being a physically more robust order than are the Ephemeroptera, it is not surprising that they were more resistant to a low dose of copper.

MDH responded positively to copper treatment. It showed an effect of copper exposure with diffusion or absence of banding in the controls and high-dose Cu^{2+} treatment. However, in the low-dose treatment the enzyme resolved into discrete bands (Fig. 1), perhaps being activated by the metal ions.

On the control portion of its gel, GK had a large dark area, probably the result of bands diffusing together, (Fig. 2). Similar dark areas developed on the α-GPD (data not shown) gel, however, on that gel the dark areas occured in the low-dose area as well. This indicated that exposure to Cu^{2+} ions is disruptive to GK and α-GPD in high concentrations, and to GK in low concentrations as well.

In GPI, there was only a difference of one thin band appearing in the untreated controls (Fig. 3); this did, however, indicate that the difference may have been biochemical, rather than metabolic, since the treated controls were homogenized when the copper was added. This was the only instance in which the treated controls showed a difference from the untreated controls. As occasionally happens, some of the lanes smeared and streaky artifacts occurred. This is not uncommon in starch-gels, and does not interfere with resolution of the bands. There was no indication that copper ions played a role in the formation of these artifacts.

Scattered bands developed in control and high-dose experimentals in IDH, with only a single low-dose developing bands (data not shown). The indication is that, the IDH enzyme was denatured by Cu^{2+} in the low dose animals; with high-dose animals

dying before this could be fully repressed. Since IDH is a necessary enzyme in the tricarboxylic acid cycle (Lehninger 1975), it is unclear why bands did not develop in all of the controls.

PEP was altered or absent in copper-treated animals. It had a few scattered bands that appeared in controls and low-dose animals but were absent in high-dose animals (not shown); most of the PEP bands, however, were generally unaffected.

SOD (Fig. 4) and MDH^+ (Fig. 5) were unaffected by Cu^{2+}. Polymorphisms in the enzymes of Odonata are known (Harrison, *et al.* 1994). Although polymorphic, EST showed no variation that could be linked to copper treatment (Fig. 6). Likewise, ALD, GAM, and XDH showed either no variation, or none attributable to exposure to Cu^{2+}. Since copper is an essential element, there was the possibility of at least some homeostatic regulation within the organisms (Luoma 1983). Also, some of these enzymes *(e.g.,* SOD) have forms that contain copper (Sanders, *et al.* 1993), which might explain its lack of effect on them under these test conditions.

Many of the bands that developed in this study were similar to those found in the damselfly study by Schott and Brusven (*op. cit.*), where bands from various enzymes were altered by exposure to different thermal regimes. Some bands diminished or disappeared altogether from the experimental lanes, while other experimental animals showed bands not present in the controls.

Exposure to high levels of Cu^{2+} had generally similar, although more rapid, effects on the naiads than did low-level exposure. On occasion, however, control and high-dose experimentals produced similar banding patterns. In part, this may have been because the animals exposed to a higher concentration of Cu^{2+} died quickly, before the copper had time to cause metabolic effects on their enzymes. The treated controls, used in testing to determine whether copper was affecting the enzymes metabolically or biochemically, showed effects in only one enzyme, GPI, where a third, fast-moving band, appeared only in the untreated controls. From this it may be deduced that copper effects are generally metabolic in origin, and that the toxicity involved is a result of disruption of metabolic processes.

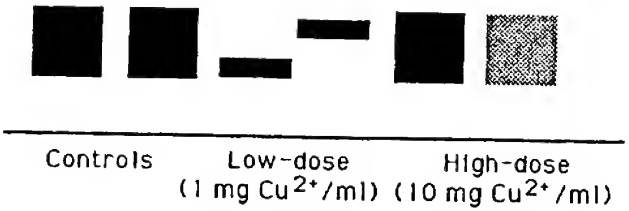

Figure 1. Representative bands from the MDH^- gel. The low-dose animals, in the center, were resolved into discrete bands, although they had different mobilities. Both the controls and the high-dose experimentals were diffuse and sometimes faint, as in the band on thye far right. Each lane represents a single animal.

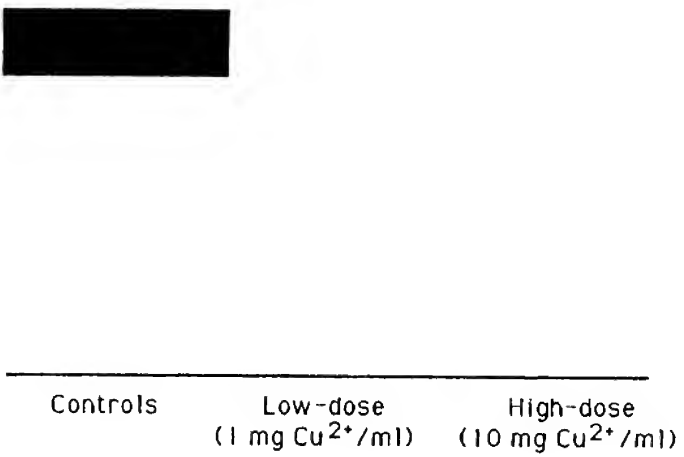

Figure 2. The large, fast-moving dark area on the control side of the GK gel was the result of bands diffusing into one another. A similar pattern appeared on the GPD gel, but included the low-dose animals as well.

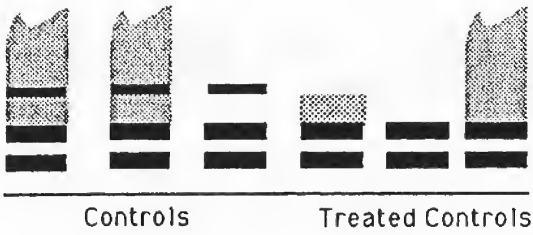

Figure 3. The thin, fast-moving bands in the GPI controls were not present in any of the copper-treated animals from either concentration of $CuSO_4$ nor from the treated controls. As shown, some bands in both treatments unaccountably smeared, but there was no indication that this was a result of copper treatment. All experimental animals exhibited banding patterns similar to the treated controls. Each lane represents a single animal.

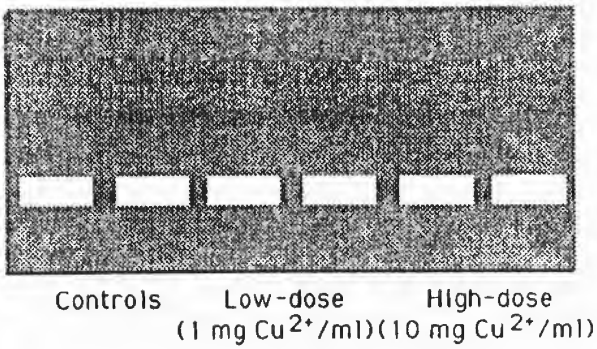

Figure 4. Representative bands from the SOD gel. No differences were seen between controls and any of the experimentals. The SOD bands remained clear, while the rest of the gel stained. Each lane represents a single animal.

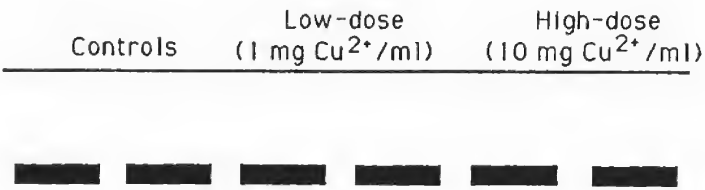

Figure 5. Representative bands from the MDH^{+} gel. No differences were seen between controls and experimentals. MDH^{+} is cationic and migrated twoard the negative pole. Each lane represents a single animal.

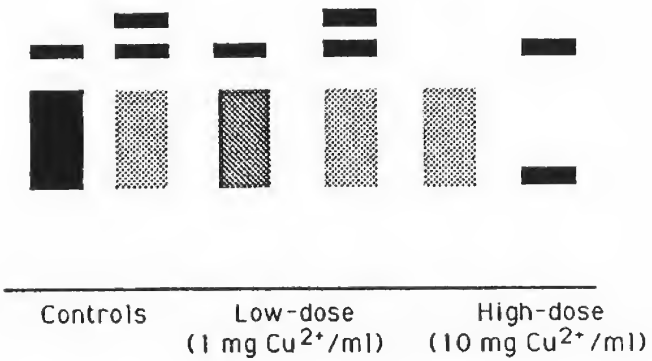

Figure 6. Representative bands from the EST gel. There was considerable variation in staining among the different lanes, but it indicated only that EST was highly polymorphic. All patterns were found among all treatments. Therefore, none of the differences could be attributed to contact with cupric ions. Each lane represents a single animal.

Summary and Conclusions

Exposing Odonata naiads to Cu^{2+} had a number of different effects on the various enzymes studied. Some enzymes were activated, others were totally unaffected, and some were denatured. That 1 mg/ml of $CuSO_4$ was sufficient to induce deleterious effects in the exposed naiads is indicative of its potency as a possible environmental pollutant in aquatic systems, even in minimal concentrations. The extensive loss of enzymatic activity should have deleterious effects on metabolism (Rusting 1992). The electrophoretic method of analyzing these effects demonstrated that it may be a more sensitive method than the traditional biotic indices for measuring water quality, since apparently visibly healthy animals may become significantly biochemically impaired by the effects of $CuSO_4$.

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Baseline Survey of Dragonflies and Damselflies of Nassawango Creek, Maryland [#]

Richard L. Orr [1]

Abstract. Nassawango Creek is a 15-mile long protected riparian corridor of bald cypress forest. Its location in Wicomico and Worcester Counties in Maryland makes the Nassawango Creek Preserve one of the most northern intact bald cypress forests in North America. Under the auspices of the Maryland Department of Natural Resources and the Nature Conservancy, the Nassawango Creek Preserve was surveyed for Odonata. Thirty-eight species of dragonflies and damselflies were observed. Their abundance, distribution, and larval habitat preferences were recorded.

Materials and Methods

The baseline survey concentrated from Nassawango Creek at Mount Olive Church Road south to where the Creek crosses Nassawango Road. The field work was conducted in June and August of 1993. The locations chosen for sampling attempted to incorporate as many different aquatic habitats as could be identified along Nassawango Creek. The nine sampled locations (listed in order, from north to south) are listed in Table 1, below.

Table 1: Locations Sampled.

L.(1): Nassawango Creek at Mount Olive Church Road
L.(2): Powerline Right-of-Way at the Voltage Station on Mount Olive Church Road
L.(3): Nassawango Creek at Snow Hill Road
L.(4): Nassawango Creek at Furnace Town Village
L.(5): Sandy Area (Parking Lot), Southwest Corner of Milville and Furnace Road
L.(6): Milville Road (Forested Section) Just South of Furnace Road Intersection
L.(7): Nassawango Creek at Red House Road
L.(8): Marshy Area Near Creek at Nassawang Sand Road Just South of Scotty Road Intersection
L.(9): Nassawango Creek at Nassawango Road

[#] This article was critically reviewed by two annonymous peers and at least one member of the MES Editorial Committee.

[1] 5215 Durham Road, East; Columbia, MD 212044.

Note: After this paper was submitted for publication, a male *Gomphaeschna antilope* (Hagen) was photographed by Dave Czaplak on July 1, 1994, at Nassawango Creek.

Results

Table 2 records the dragonflies and damselflies observed at Nassawango Creek along with the location and relative abundance of each species.

Table 2: Species, Locations and Relative Abundances of Odonata.

Species	Common Name	Location	Abundance
AESHIDAE			
Anax junius (Drury)	Common Green Darner	L(8)	Uncommon (J)
		All	Common (A)
Boyeria vinosa (Say)	Fawn Darner	L(1)	Common (A)
Epiaeschna heros (Fab.)	Swamp Darner	All	Common (J)
		All	Uncommon (A)
Nasiaeschna pentacantha (Rambur)	Cyrano Darner	L(4)	1 seen (J)
GOMPHIDAE			
Arigomphus villosipes (Selys)	Unicorn Clubtail	L(9)	Uncommon (J)
Gomphus exilis Selys	Ashy Clubtail	All	Abundant (J)
Hagenius brevistylus Selys	Dragonhunter	L(1,4,9)	Common (A)
		L(2,8)	Uncommon (A)
Stylurus plagiatus (Selys)	Russet-tipped Clubtail	L(9)	Common (A)
MACROMIIDAE			
Didymops transversa (Say)	Stream Cruiser	L(3,4)	Common (J)
		L(7)	Uncommon (J)
Macromia illinoiensis georgina (Say)	Illinois River Cruiser	L(9)	Uncommon (J)
		L(9)	Uncommon (A)
CORDULIDAE			
Epitheca princeps Hagen	Prince Baskettial	L(6,8)	Uncommon (J)
		L(8,9)	Uncommon (A)
Epitheca cynosura (Say)	Common Baskettail	L(8)	Common (J)
		L(9)	Uncommon (J)
Somatochlora linearis (Hagen)	Mocha Emerald	L(6)	Uncommon (J)
		All	Common (A)
LIBELLULIDAE			
Erythemis simplicicollis (Say)	Eastern Pondhawk	L(9)	Common (J)
		All	Common (A)
Libellula cyanea Fab.	Spangled Skimmer	L(4,6,9)	Uncommon (J)
		L(8)	Uncommon (A)
Libellula incesta Hagen	Slaty Skimmer	L(4,9)	Uncommon (J)
		L(1,4,8)	Common (A)
		L(9)	Abundant (A)

Libellula lydia Drury	Common Whitetail	All	Common (J)
		All	Common (A)
Libellula needhami Westfall	Needham's Skimmer	L(8,9)	Uncommon (J)
		L(8,9)	Common (A)
Libellula pulchella Drury	Twelve-spotted Skimmer	L(2)	1 seen (A)
Libellula semifasciata Burmeister	Painted Skimmer	L(4,8,9)	Uncommon (J)
		L(2)	1 seen (A)
Libellula vibrans Fabricius	Great Blue Skimmer	L(3,4,7)	Common (J)
		L(6,8,9)	Uncommon (J)
		L(1,4,8,9)	Common (A)
Pachydiplax longipennis (Burmeister)	Blue Dasher	L(3,4)	Uncommon (J)
		L(9)	Common (J)
		All	Abundant (A)
Pantala flavescens (Fab.)	Wandering Glider	L(8)	1 seen (A)
Pantala hymenea (Say)	Spot-winged Glider	L(8)	1 seen (A)
Perithemis tenera (Say)	Eastern Amberwing	L(9)	Abundant (A)
Tramea carolina (L.)	Carolina Saddlebags	L(9)	1 seen (A)
Tramea lacerata Hagen	Black Saddlebags	L(9)	1 seen (J)
CALOPTERYGIDAE			
Calopteryx maculata (Beauvois)	Ebony Jewelwing	L(3,4,7)	Abundant (J)
		L(6,8,9)	Common (J)
		L(1,4,8,9)	Common (A)
LESTIDAE			
Lestes rectangularis Say	Slender Spreadwing	L(4)	Uncommon (A)
COENAGRIONIDAE			
Argia tibialis (Rambur)	Blue-tipped Dancer	All	Abundant (J)
		L(1,4)	Uncommon (A)
Chromagrion conditum (Hagen)	Aurora Damsel	L(4)	1 seen (J)
Enallagma civile (Hagen)	Civil Bluet	L(4)	1 seen (A)
Enallagma exsulans (Hagen)	Stream Bluet	L(3)	Uncommon (J)
Enallagma geminatum Kellicott	Skimming Bluet	L(3,9)	Uncommon (J)
		L(4)	Common (J)
Enallagma signatum (Hagen)	Orange Bluet	L(9)	Common (J)
		L(4)	Common (A)
		L(9)	Abundant (A)
Ischnura posita (Hagen)	Fragile Forktail	L(3,8)	Uncommon (J)
		L(4,7)	Common (J)
		L(9)	Abundant (J)
		All	Common (A)

Ischnura ramburii (Selys)	Rambur's Forktail	L(3,4,7)	Uncommon (J)
		L(9)	Abundant (J)
		L(9)	Uncommon (A)
Ischnura verticalis (Say)	Eastern Forktail	L(9)	Abundant (J)
		L(9)	Common (A)

(J) = Recorded in June
(A) = Recorded in August
Abundant = 50+ seen per day per Location
Common = 10 to 50 seen per day per Location
Uncommon = 10 or less seen per day/Location

Based on the known biology of the adult Odonata recorded at Nassawango Creek Preserve and a limited larval survey, the preferred larval habitats are either known or can be inferred. Some species are generalists while others are more specific in their habitat requirements. Therefore, the more general a species is in its larval requirements the more different habitats it will be listed under. Table 3 presents the different Nassawango Creek Preserve aquatic habitats and their associated Odonata larvae composition (as observed or inferred).

Table 3: Odonata Species Listed by Preferred Larval Habitat

Habitat 1: Nassawango Creek and Tributaries - Narrow Channel; Mostly Shaded; Little Emergent Vegetation (Northern Part of Preserve).

Boyeria vinosa
Gomphus exilis
Somatochlora linearis
Libellula vibrans
Calopteryx maculata
Enallagma exsulans
Enallagma signatum
Hagenius brevistylus
Didymops transversa
Libellula incesta
Pachydiplax longipennis
Argia tibialis
Enallagma geminatum
Ischnura posita

Habitat 2: Nassawango Creek - Wide Channel; Open (Southern Part of Preserve).

Anax junius
Hagenius brevistylus
Stylurus plagiatus
Epitheca cynosura
Erythemis simplicicollis
Libellula incesta
Libellula needhami
Pachydiplax longipennis
Arigomphus villosipes
Gomphus exilis
Macromia illinoiensis georgina
Epitheca princeps
Libellula cyanea
Libellula lydia
Libellula vibrans
Perithemis tenera

(**Habitat 2**: Continued)

Tramea carolina
Argia tibialis
Enallagma signatum
Ischnura ramburii
Tramea lacerata
Enallagma geminatum
Ischnura posita
Ischnura verticalis

Habitat 3: **Ponds (Impoundments of, or Side Ponds Next to, Creek - - Sunlit).**

Anax junius
Erythemis simplicicollis
Libellula incesta
Pachydiplax longipennis
Tramea lacerata
Chromagrion conditum
Enallagma geminatum
Ischnura posita
Epitheca cynosura
Libellula cyanea
Libellula lydia
Tramea carolina
Lestes rectangularis
Enallagma civile
Enallagma signatum
Ischnura ramburii

Habitat 4: **Swamp Environment (Flood Plain Areas of Creek, with Little or No Water Flow)**

Epiaeschna heros
Somatochlora linearis
Libellula vibrans
Lestes rectangularis
Nasiaeschna pentacantha
Epitheca cynosura
Pachydiplax longipennis
Ischnura posita

Habitat 5: **Marsh Environment (Flood Plain Areas of Creek, With Little or No Water Flow).**

Anax junius
Erythemis simplicicollis
Libellula lydia
Libellula semifasciata
Perithemis tenera
Tramea lacerata
Enallagma civile
Enallagma signatum
Ischnura ramburii
Libellula cyanea
Libellula incesta
Libellula needhami
Pachydiplax longipennis
Tramea carolina
Lestes rectangularis
Enallagma geminatum
Ischnura posita
Ischnura verticalis

Habitat 6: **Temporary Rain or Irrigation Pools in Open Fields.**

Libellula pulchella
Pantala hymenea
Pantala flavescens
Tramea lacerata

Habitat 7: Permanent Irrigation Ponds on Agricultural Land.

Anax junius
Libellula incesta
Libellula needhami
Tramea carolina
Enallagma civile
Ischnura verticalis
Erythemis simplicicollis
Libellula lydia
Pachydiplax longipennis
Tramea lacerata
Ischnura posita

Discussion:

A comprehensive Odonata survey would require repeated sampling from March through November over an extended number of years. However, this paper does provide a list of those species that naturalists and other scientists are most likely to encounter in future limited surveys or biodiversity studies of the creek. The list also provides a baseline against which to measure future changes in water quality or habitat composition, which would manifest themselves in changes in the numbers and relative abundances of Odonata species.

The dense shade and dark-colored water of Nassawango Creek has not promoted the luxuriant growth of aquatic plants. This has reduced the biodiversity of aquatic insects present. In addition, the lack of clear sunlit ponds, free from the runoff from agricultural fields, has further reduced the number of Odonata species that could occur in the Nassawango Creek area. Conspicuously absent from this list of Odonata were species belonging to the genera *Celithemis* and *Sympetrum*, which are normally common in healthy pond and marsh environments throughout Maryland's coastal plain.

Where the creek was exposed to the sun (usually due to a road crossing) or in an open adjacent marshy or swampy area buffered from the nearby agricultural fields, the biodiversity of Odonata was noticeably greater. At Nassawango Creek Preserve those historical Odonata species whose larvae utilize the creek have probably been more successful in maintaining their populations than those species which required healthy marshes and ponds.

A Late Season Oviposition Record for the Viceroy, *Limenitis archippus* (Cramer) [Lepidoptera: Nymphalidae] in Southern Maryland#

A.P. Platt[1]

On 26 October, 1996, I had occasion to visit the Calvert Marine Museum (CMM) on Solomons Island in Calvert County, MD. While standing beside the outdoor otter enclosure at the Museum, I spotted a large orange butterfly circling around several six- to eight-foot high willow shrubs (*Salix* spp.) located beside a small wooden footbridge. At first, I thought that the butterfly was a late-migrating monarch, *Danaus plexippus* (L.), but closer inspection revealed that it was a female viceroy *Limenitis (Basilarchia) archippus* (Cramer) in worn condition.

I watched as the butterfly laid three eggs on the upper tips of three separate leaves. Then, as she was backing down a fourth leaf, preparing to oviposit, I caught her with my hand, without injuring her. The female was observed ovipositing on this unseasonably warm, overcast day, about 3 P.M. E.D.T., with the temperature estimated to be about 60°F (15.6°C). I collected all three of the eggs she had laid, as well. Each had been placed on the dead brownish-colored tip of each willow leaf, the leaf tips themselves having succumbed to earlier frosts. (A nearby willow shrub already had dropped all of its leaves). I believe that the willows were *Salix fragilis* (L.), but this requires further confirmation. (At least the shrubs all had very delicate branch nodes, which easily snapped off under slight finger pressure).

The female was placed sequentially in several different sized (small-to-large) oviposition chambers in my laboratory at UMBC. These contained both cuttings and potted plants of weeping willow, *Salix babylonica* (L.); but she laid no additional eggs while in captivity. She lived for more than seven days, but spent most of her time resting on the moist soil at the bottom of the Longday photo-chambers. She did not fly much at all during her captivity.

All three of her eggs collected in Calvert County hatched, but the larvae soon died because the leaves of the willow cuttings, on which they had been placed, had dried up before the larvae hatched. It is doubtful that these larvae could have fed and grown to third instar (which they must do before they can form hibernacula, and then diapause) before the onset of severely cold and freezing fall temperatures occurred, had they been left in the wild. Such growth requires a minimum of two weeks, even at constant room temperature in the laboratory. Their growth rates in the wild would have been much slower than this during the first several weeks of November because of the colder outdoor temperatures.

This note was critically reviewed by two annonymous peers and at least one member of the MES Editorial Committee.

[1] Professor, Department of Biological Sciences, 1000 Hilltop Circle, University of Maryland - Baltimore County (UMBC), Baltimore, MD 21250, USA.

Key words: Oviposition, *Limenitis archippus*, Nymphalidae

I have previously described the oviposition behavior of female admirals (Platt 1979). This female specimen represents either a very late third generation, or possibly even a fourth generation of Viceroys for the 1996 growing season. Such late season individuals show a greater propensity to cross-hybridize with individuals of *Limenitis arthemis astyanax* (Fab.), during the September - November time period, as judged by the capture dates of many of the wild F_1 hybrid "rubidus" Stkr. specimens (Platt, *et al.* 1978; Platt 1983; Covell 1994; Platt and Maudsley 1994). The wild-caught female specimen has been labeled and placed in the UMBC collection.

Larval diapause among viceroys is facultative (Clark and Platt 1969), and is under polygenic control (Hong and Platt 1975). Larvae from localities differing in latitude exhibit differences in the photoperiod thresholds which induce diapause. Diapausing larvae lose water, and actively synthesize glycerol (as an anti-freeze) when exposed to sub-freezing temperatures (Frankos and Platt 1976). However, certain individuals from southern Florida apparently lack larval diapause capabilities altogether, even when the annual photophase is of shortest duration (Williams and Platt 1987; Flaim and Platt 1998, *in review*). Both the second instar and the early third instar (pre-diapausing) viceroy larvae exhibit diapause responses to very dim light (±0.10 foot candles), and they even respond to "black-light" (Platt 1984; Platt and Harrison 1988). Finally, the observation has been made that diapause initiation correlates with the appearance of the pale (whitish) dorsal abdominal saddle-patch on the second and early third instar viceroy larvae. This larval saddle-patch is centered above the fifth abdominal segment, and it overlies both segmentally arranged paired abdominal ganglia and the developing (paired) larval gonads. Among the early stage pre-diapause larvae this saddle-patch helps the larvae to closely resemble small bird-droppings. In earlier papers, I have made the suggestion that this pale translucent area possibly may be photosensitive. Earlier experiments revealed that although diapause initiation could not be prevented by "masking" this area, adverse affects were produced on further larval growth and metamorphosis among the experimental animals, but not among the controls (Platt 1989).

Acknowledgement

I wish to thank Colleen Wilkins of the Biological Sciences Department, UMBC, for typing this article.

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Contents

Cover Illustration

The type specimens of the new subspecies,
Incisalia henrici viridissima Pavulaan,
the "Greenish Henry's Elfin."
(ventral view: Male - above, Female - below)

(Photographs taken by Harry Pavulaan.)